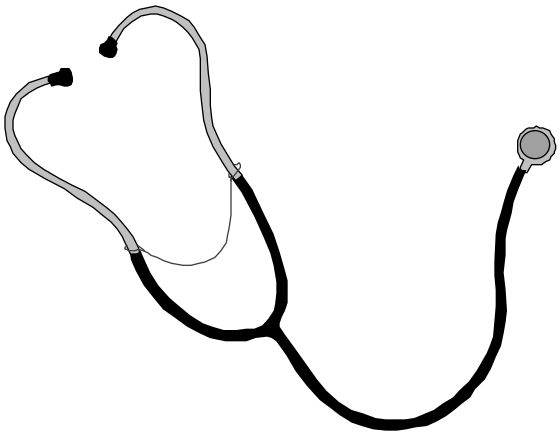
Activity 5

How Hazardous Substances Affect People



Duration	2 class periods
Grade Level	7-10
Key Terms/ Concepts	Adverse Health Effects Epidemiological Exposure Toxicology
Suggested Subjects	Biology Chemistry Life Science

Purpose

This activity helps students gain an appreciation for how scientists determine the human health effects of hazardous substances. Students also demonstrate how hazardous substances can affect the health of test animals.

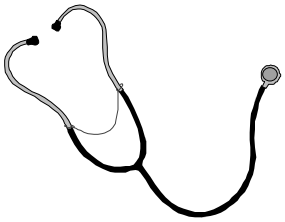
Note: This activity involves the exposure of worms to a hazardous substance. Some students may object to this on ethical or moral grounds.

Background

Toxicology is the study of the effects of poisons on living organisms. Scientists conduct a variety of studies to discover toxicological information about hazardous substances. Two of the most common types of studies are (1) **epidemiological** studies—matching disease and other **adverse health effects** in humans with possible causes—and (2) animal toxicological studies.

The Federal government's Superfund Program, administered by the U.S. Environmental Protection Agency (EPA), helps protect people and the environment by cleaning up hazardous waste sites. Well-designed, properly controlled epidemiological studies conducted at or near hazardous waste sites can provide information important in making cleanup decisions.

On their own, these studies are not always conclusive. This is primarily because it is difficult to determine the exact amount of the chemical or chemicals contaminating the site to which human populations have actually been exposed (had contact with). Many times health histories are incomplete, and potentially exposed populations are too small for statistical analyses. In addition, many uncontrolled variables—such as genetics, exercise, diet, or cigarette use—may complicate detecting the effects of the hazardous substances.



When epidemiological studies cannot be done, well-designed animal studies can provide a wealth of information. This information can be used to predict potential effects in humans over a range of **exposure** levels—from **acute,** a single exposure to a hazardous material for a brief length of time, to **chronic,** continuous or repeated exposure to a hazardous substance over a long period of time.

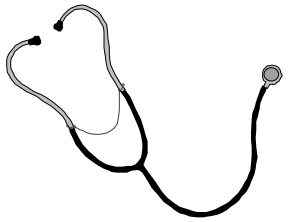
For more information on health effects caused by hazardous substances, see the Suggested Reading list found at the end of the Haz-Ed materials. Other Haz-Ed materials that are related to this topic include *Fact Flash 1: Hazardous Substances and Hazardous Waste; Fact Flash 9: Common Contaminants;* and *Activity 6: Examining the Effects of Pollution on Ecosystems.*

Preparation

1. Assemble the following materials:

   - Small plastic cups (5 per group)
   - Three empty one-liter plastic soft drink containers with caps
   - Refrigerated tap water
   - Automobile antifreeze (ethylene glycol)
   - Live, fresh-water black (hair) worms, sold as fish food in pet stores (50 or more worms per group)
   - 16-ounce measuring cup
   - 1-ounce measuring cup (used to measures doses of cough syrup)
   - plastic wrap
   - Tape and markers
   - Copies of the Student Handout, *Black (Hair) Worm Experiment* (1 per group)
   - Copies of the Student Worksheet, *Black (Hair) Worm Experiment* (5 per group)

   NOTE: Fresh-water hair worms are inexpensive, easy to see because of their dark color, and quite active. They survive best in a small amount of refrigerated water (they die if submerged) and should be kept in the refrigerator until class time. If washed every day, they can live 1 to 2 weeks in the refrigerator. If worms are not available, you may substitute some other fresh water invertebrate, which can be obtained at tropical fish or pet stores. Brine shrimp, available at some pet stores, also may be substituted for fresh water invertebrates, but you will need to add table



salt at a 5 percent solution in the water before adding the shrimp.

2. Fill each of the three 1-liter containers (they must be clean) with 16 ounces of water and clearly mark the 16-ounce level on the side. Pour out the water and shake the containers dry.

3. Label the containers either Low (6 percent), Medium (12 percent), or High (24 percent) ethylene glycol. For the low-dose solution pour 1 ounce of antifreeze in the container marked Low (6 percent) and fill up to the 16-ounce mark with water. For the medium-dose solution pour 2 ounces of antifreeze in the container marked Medium (12 percent) and fill up to the 16-ounce mark with water. For the high-dose solution pour 4 ounces of antifreeze in the container marked High (24 percent) and fill up to the 16-ounce mark with water. Shake or stir well. *(NOTE: Any substance can be toxic in a high enough concentration. Handle the antifreeze carefully.)*

4. Contact your local sanitation or health department to request information on the proper disposal methods for antifreeze in your community. Can it be poured down the drain? Is there a recycling center for this type of substance?

Procedure

1. Using the information in the Background section, discuss how scientists conduct studies to get information on toxic substances.

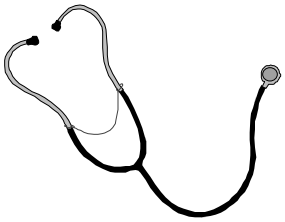
2. Divide the class into teams of two to four students each. **Caution students to handle the antifreeze carefully.**

3. Provide each team with five clean plastic cups, tape, marker, one copy of the Student Handout, and five copies of the Student Worksheet.

4. Have the students label the first container “low dose,” the second “medium dose,” and the third “high dose.” Have them label the fourth container “control pre-test” and the fifth “control post-test.”

5. Provide each team with some live worms. Have all groups pour just enough cold water into the “control pre-test” container to barely coat the bottom. Too much water will drown the worms.

6. Have the students place about 10 worms in the water and watch for any behavioral changes, recording the results at the end of 4 minutes on the Student Worksheet. Have them leave the worms in the container.



7. Have teams pour just enough water-antifreeze solution into each container to barely coat the bottom, using the solutions you prepared in advance. Have half of the teams start with the "low dose" container first and proceed in order to the "high dose." Have the other teams start with the "high dose" container first and proceed in order to the "low dose" container.

8. Have the students conduct each test one at a time, using different worms for each container (about 10 per container). Remind teams to record their observations for each test on the Student Worksheet.

9. Have all groups end the experiment by pouring just enough cold water to barely coat the bottom of the "control post-test" container. *(NOTE: Control observations at the beginning and end of the experiment are intended to help rule out effects not related to the antifreeze, such as water temperature.)*

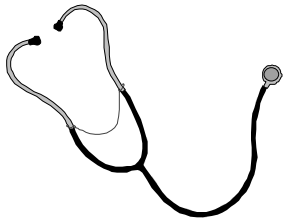
10. After the experiments, have each group describe the worm behavior they observed during each test and discuss the answers to the questions on the Student Worksheet.

11. Have students dispose of the antifreeze solutions properly, according to the information you received from your local sanitation or health department.

Extensions (Optional)

- Have the students plan and conduct an experiment to determine if there is a concentration of antifreeze and water that does not appear to change the behavior of the worms over a 24- or 48-hour period. The purpose is to determine if there is a threshold for an acute (rapid) effect; in other words, a level of exposure below which there is not likely to be an adverse health effect in the short-term. The students' experimental plan should at the very least include an appropriate control group, a sufficient number of worms, observation procedures, and an explanation of the experimental conditions, including procedures for rinsing the worms once a day, cleanliness, covering containers to prevent evaporation, and refrigerator temperatures.

- Have the students search the library for information on worm biology. Focus their attention on worm anatomy and physiology, function in ecosystems, and whether the adverse effects of antifreeze on worms might be compared to the potential effects of antifreeze on human health or ecosystem health.



Teacher's Answer Key — Black (Hair) Worm Experiment

(1) Were there obvious behavioral differences between the control groups and the antifreeze-exposed groups? If yes, describe.

There should be differences in mobility even with only 10 animals per group. After about 10 minutes nearly all worms exposed to the antifreeze solutions probably will be dead. The higher the concentration of antifreeze the faster they die. Also, the smaller the worms (young worms) the faster they die. In the unlikely event there are no differences between treated and control groups, perhaps more worms per group are needed, or the antifreeze concentration is too low to cause an observable effect, or the worms are not susceptible to the adverse effects of antifreeze. All of these possibilities could be tested in another experiment if materials allow.

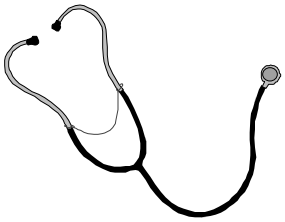
(2) Did the concentration of antifreeze in the water influence the degree of behavioral change? If yes, describe.

After an initial increase in activity, you should find that the higher the dose, the more quickly the worms' mobility decrease. The degree and severity of toxic effects are primarily a function of dose (the amount of contact or exposure to the chemical). However, many other factors including differences in susceptibility among individuals within a species also influence the outcome. Because humans manifest an unusual degree of individual variability, large numbers of people must be similarly exposed to clearly demonstrate that a chemical causes a specific toxic effect. Using animal toxicity studies to determine the potential adverse effects of specific substances has many advantages. Genetically similar individual animals can be used in relatively large numbers and exposures can be controlled over a range of dose levels. The results of animal toxicological studies are used to predict potential effects in humans at dose levels relevant to possible human exposures.

(3) Was there a safe level of exposure? In other words, was there an antifreeze solution that did not appear to cause an effect over the 4-minute observation period? How could you tell?

Ideally, at least one dose level in an experiment should have an observable effect different from the others during the observation period. That is, if all the doses cause the same reaction, you have only learned that antifreeze has an effect at a concentration equal to or greater than the lowest dose used. You have not determined the minimum concentration that will cause an effect, or the maximum concentration that has no effect.

If all three doses in your experiment caused the same reaction during the 4-minute observation period, you may want to repeat the experiment using a lower concentration. For example, you could prepare a 3 percent solution by pouring 1/2 oz. of antifreeze into one of the liter containers you used earlier, and filling it up to the 16 oz. mark with water. Then repeat the experiment using this solution and observe the reaction. Are the results different after 4 minutes? How about after the total time that elapsed during the other experiment?



(4) Does the acute (rapid, short-term) effect of antifreeze on the worms indicate anything about what the long-term or chronic (lifetime) effects might be?

No. Long-term or chronic exposures to hazardous substances frequently result in different effects from those observed after only a brief exposure. This makes the effects of long-term exposure more difficult to link to a specific cause. Reproductive organs may be affected; mutations in cell structure, liver damage, and so forth may not show up until the next generation.

(5) Is behavior the only possible measure of effect? Why or why not?

Although behavior is an inexpensive indicator of a potentially fatal adverse effect, it is not the only measure of effect. It is used in this experiment because it does not require sophisticated equipment to study.

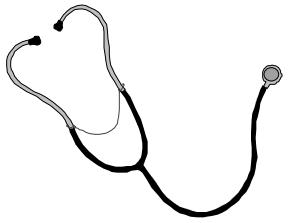
(6) Can you determine from this experiment the cause of death of the worms?

No. Damage could have been done to vital organs such as the liver and kidney, which would subsequently make the worms too sick to move, or the antifreeze could have directly affected their neuromuscular system, brain, sensory systems, and other organs, thereby slowing their mobility.

Hazardous substances adversely affect living organisms through a variety of mechanisms, many of which are not yet known. Some chemicals alter DNA, damage DNA repair mechanisms, or destroy cells by damaging their membranes, interacting with cell receptors, depleting substances essential to cell survival, or inhibiting production of vital enzymes. Some potentially hazardous substances are not hazardous until the body breaks them down (or metabolizes them) into substances that are toxic. For example, carbon tetrachloride is broken down in the liver to a highly reactive chemical that initiates a chain reaction which destroys a crucial liver cell enzyme system (cytochrome P-450).

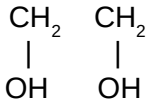
(7) Is it possible that while antifreeze affects the health of worms, it has no effect on humans? Is it also possible that antifreeze has no effect on worms but adversely affects human health? If yes, describe.

The answer to both is yes. However, epidemiological studies and accidental poisonings verify that antifreeze causes serious and often fatal effects when ingested by humans. The liver breaks down ethylene glycol into aldehydes, glycolate, oxalate, and lactate that may initially cause nausea, seizures, respiratory failure, coma, and cardiovascular collapse. Survivors of the acute phase ultimately exhibit kidney failure, severe acidosis (lowered blood pH), and low blood calcium levels. The fatal kidney damage results mainly from the formation of oxalate-calcium crystals that precipitate in the kidney tubules. These changes may also occur in the liver, heart, blood vessels, and brain. In addition, the aldehydes, glycolate, and lactate acidify the blood to dangerous levels.



Black (Hair) Worm Experiment

The purpose of this experiment is to determine the potential effect of three concentrations of antifreeze (ethylene glycol) on fresh-water worms. There are five steps.



ethylene glycol

Your teacher will provide you with:

- Five small, clean plastic cups
- Live, fresh-water black worms (hair worms)
- Tape and markers
- Five copies of a Student Worksheet for recording your observations
- Antifreeze (ethylene glycol) solutions of 6 percent, 12 percent, 24 percent
- Refrigerated tap water.

Step 1 Label one cup “low dose,” the second cup “medium dose,” the third cup “high dose.” Label the fourth cup “control pre-test” and the fifth cup “control post-test.”

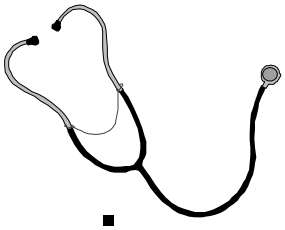
Step 2 Add just enough cold tap water to barely cover the bottom of the container marked "control pre-test," and place about 10 worms in the water. ***(DO NOT SUBMERGE THE WORMS: THEY WILL DIE.)*** Observe the worms for 4 minutes and watch for any changes in their behavior. Record the results on the appropriate line of the Worksheet. Set the “control pre-test” cup aside, but do not throw it away.

Step 3 After recording the behavior of the “control pre-test” group, conduct similar observations of different worms (about 10 per cup) in order from “low dose” to “high dose” or from “high dose” to “low dose” depending on your instructor’s directions. Use the appropriate antifreeze mixture prepared by your instructor for each dose level. Use the Worksheet to record the behavior of each group of worms at the end of 4 minutes.

Step 4 After you have observed the results from all 3 solutions, repeat the control test by again adding barely enough cold water to cover the bottom of the cup labeled “control post-test,” and place about 10 worms in the water. Observe for 4 minutes for any behavioral changes. Record the results on the Worksheet.

Step 5 At the end of the experiment observe the total time and take one last look at the worm behavior in all of the cups.

Step 6 Answer the questions on the Worksheet.



Black (Hair) Worm Experiment

Test	Behavior after 4 minutes	Behavior at end of experiment	Total time from start of experiment
control pre-test low dose medium dose high dose control post-test			

Answer the following questions

(1) Were there obvious behavioral differences between the control groups and the antifreeze-exposed groups? If yes, describe.

(2) Did the concentration of antifreeze in the water influence the degree of behavioral change? If yes, describe.

(3) Was there a safe level of exposure. In other words, was there an antifreeze concentration that did not appear to cause an effect over the 4-minute observation period? How could you tell?

(4) Does the acute (rapid, short-term) effect of antifreeze on the worms indicate anything about what the long-term or chronic (lifetime) effects might be?

(5) Is behavior the only possible measure of effect? Why or why not?

(6) Can you determine from this experiment the cause of death of the worms?

(7) Is it possible that while antifreeze affects the health of worms, it has no effect on humans? (Is it also possible that antifreeze has no effect on worms but adversely affects human health?) If yes, describe.